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Big Spider, Big Genome: Chromosome-Level Genome of a North American Tarantula (*Aphonopelma marxi*) and Comparative Genomics Across 300 Million Years of Spider Evolution

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Abstract

The comparison of chromosome-level genomes allows biologists to investigate new axes of organismal evolution. Spiders comprise a significant proportion of known arachnid diversity, with many complex morphologies and unique natural histories, yet comparative genomics in spiders has been limited due to the number of available genomes. We present a de novo chromosomal reference genome of a mature male tarantula, *Aphonopelma marxi*, and comparatively examine spider genome evolution across the order Araneae. Using PacBio HiFi and Hi-C sequencing, the final 6.5 Gb assembly consists of 17 autosomes, 1 X chromosome, and 127 unplaced scaffolds, with an N50 of 370 Mb and Arachnida (odb10; 2,934 genes) BUSCO of 96.7%. By comparing 20 additional spider genomes from 15 families, we find mygalomorphs (trapdoor spiders and their kin) generally possess more repetitive genomes with similar composition compared to their much more diverse sister lineage, the araneomorphs. We report mygalomorphs recover a lower number of completed BUSCOs than araneomorph spiders, a finding not correlated with sequencing coverage, as mygalomorphs have a portion of missing or derived BUSCOs in the current arachnid dataset. Across the Araneoidea (orb-weaving spiders and their kin), there is a correlation between decreasing genome size and repeat content, suggesting repetitive elements are being lost or removed. Importantly, visualization of macrosynteny across available genomes highlights structural rearrangements and allows identification of previously unreported sex chromosomes. This new, high-quality mygalomorph genome will provide new avenues of exploration for arachnid evolutionary biology.

Key words: arachnid, Theraphosidae, de novo assembly, PacBio HiFi, synteny, repeat content.

Significance

The limited availability of spider chromosome-level genomes leaves much room for investigating how their genomes evolved over their 300-million-year existence. Our de novo assembly is the first chromosomal genome of the North American tarantula genus *Aphonopelma*. We compare this genome with other available spider genomes, revealing high repeat content, and identify homologous chromosomes across the Araneae. This is the first study to provide a wider context on spider genomic evolution and produces a chromosomal reference genome for the most well-studied tarantula lineage.

Introduction

Arachnids have played a significant role in the evolution of the planet. They are critical to the healthy functioning of terrestrial ecosystems where they take on roles as predators, disease transmitters, and detritivores. Whether it is their long evolutionary history, their diversity of natural histories and ecologies, or their unique venom and its properties, they often grab the attention of the public. Unfortunately, as important as they are, research often falls behind similarly important taxonomic groups—this is especially true with regard to genomics (Sanggaard et al. 2014, Schwager et al. 2017, Garb et al. 2018). For example, when compared to the available genomic resources for vertebrates (Johnson et al. 2018, Olagunju et al. 2024, Stiller et al. 2024), research into arachnid genome evolution is an area of much opportunity. While advances in sequencing technologies and genomic pipelines have allowed for a number of new arachnid genomes to become available (Fan et al. 2021, Gainett et al. 2021, Fan et al. 2023, Gainett et al. 2024, Miles et al. 2024, Kulkarni et al. 2025, Schöneberg et al. 2025), there are only ~80 on NCBI datasets as of September 2025 (mostly spiders, mites, and ticks) from among the estimates of more than 110,000 described species and possibly more than 1 million species estimated to occur across the world (Kuntner 2022). There is tremendous room to investigate how arachnid genomes have changed over time and the evolutionary consequences.

As the dominant arachnid order, spiders (Araneae) are an ancient group of predators that have existed for over 300 Ma, with the oldest fossils discovered from the Carboniferous and Triassic periods (Selden and Gall 1992, Selden et al. 2014, Garwood and Dunlop 2023). The current classification recognizes three major lineages: the relatively depauperate Mesothelae (the segmented spiders), the hyper-diverse Araneomorphae (e.g. orb-weavers, wolf spiders, jumping spiders, etc.), and the often-cryptic Mygalomorphae (e.g. tarantulas and trapdoor spiders). The mygalomorphs contain some of the largest, most long-lived (more than 20 years in Rix et al. 2023, up to 43 years in extreme outlier examples in Mason et al. 2018), and most venomous spiders in the world (Gray 2010, Hedin et al. 2018, Loria et al. 2025). These spiders typically have highly conserved morphology that is hypothesized to have been caused by their limited niche space (Wilson et al. 2023) which often masks their actual species diversity and evolutionary history (Hedin et al. 2018, Foley et al. 2019, Opatova et al. 2020, Korba et al. 2022, Calatayud-Mascarell et al. 2022, Montes de Oca et al. 2022, Briggs et al. 2023, Ciaccio et al. 2024, Briggs et al. 2025, Fonseca-Ferreira et al. 2025, Wilson et al. 2025).

At the time of this study, the ~3,000 named mygalomorph species are represented by four published genomes. In 2014, the first de novo sequencing and assembly of a mygalomorph genome was attempted; however due to limitations of the sequencing technologies at the time, and the estimated large genome size of 6.5 Gb (haploid), only a highly fragmented, draft assembly of *Acanthoscurria geniculata* was possible (Sanggaard et al. 2014). Given the phylogenetic and medical importance of this lineage, having mygalomorph genomes available has been highlighted as a priority target for studying spider evolution (Garb et al. 2018). The remaining three mygalomorph genomes are classified as chromosomal and were produced in the era of accurate long-read and Hi-C sequencing: (i) a 5 Gb female tarantula (Theraphosidae) *Chaetopelma lymberakisi* from Crete (European Reference Genome Atlas, McCartney et al. 2024) and (ii and iii) two species of *Macrothele* (Macrothelidae), a type of funnel-web spider, *M. cretica* and *M. yani* have been published (You et al. 2024). Based on these samples, mygalomorph genomes are on the larger size of known arachnids, varying from 5 to 6.8 Gb (haploid). However, a thesis published by Yorke (2020) revealed that genome size across tarantulas alone varied greatly from ~2.0 to ~15.4 Gb.

Karyotype, sex chromosome number, and genome size vary dramatically in spiders. It has been reported that mygalomorph diploid karyotype numbers range from 14 to 128 (Rezac et al. 2006, Kral et al. 2011, 2013, Sember et al. 2020), with these spiders typically having an X0 sex chromosome system where males only have one copy of each X chromosome, but Y chromosomes have also been reported (Kral et al. 2013, 2022, Sember et al. 2020). Perhaps most interestingly, mygalomorph spiders range from having 1-13 X chromosomes (Kral et al. 2013). These dramatic differences in karyotype number are purportedly due to fission/fusion (Kral et al. 2013); therefore, identification of homologous regions across the group will allow us to unravel this mystery by tracking these regions across the order over millions of years of evolution. Lastly, based on the available genomes, genome size varies by almost an order of magnitude between mygalomorphs and araneomorphs (*Tetragnatha montana* = 0.7 Gb, *Macrothele yani* = 6.7 Gb); however, how the genome changes with genome size in a phylogenetic context has not been deeply investigated.

Tarantulas (Araneae, Theraphosidae) represent the most diverse family of mygalomorphs, with ~ 1,100 formally described species and a distribution that spans all continents except Antarctica (World Spider Catalog 2025, diversity and systematics reviewed in Briggs and Hamilton 2024). Tarantulas from the genus *Aphonopelma* (Pocock 1901), found in North and

Central America, are arguably the most well-studied genus of tarantulas to date. Compared to other genera, the diversity and classification of *Aphonopelma*, in particular the North American species, have been deeply investigated using molecular, morphological, and ecological data. As such, this group has robustly defined species boundaries (Prentice 1997, Hamilton et al. 2011, 2014, 2016, 2024, Hendrixson et al. 2013, Wilson et al. 2013, Graham et al. 2015, 2020, Turner et al. 2018, Hendrixson 2019) as well as documentation of their ecology and movement patterns (Shillington and Verrell 1997, Janowski-Bell and Horner 1999, Punzo and Henderson 1999, Janowski-Bell 2001, Shillington and Peterson 2002, Shillington 2005, Dundee et al. 2012, Hamilton et al. 2012, Booster et al. 2015, Blais 2022), providing valuable information that can be extrapolated to other tarantula species. More recently, the exploration of embryogenesis in *Aphonopelma hentzi* has provided foundational knowledge for mygalomorph and spider development (Setton et al. 2019, 2024). Given the wealth of evolutionary knowledge accumulated for this genus, they have been established as an excellent model for further genomic investigation.

Herein, we present a high-quality de novo chromosomal reference genome of a male *Aphonopelma marxi* (Simon 1891), comprising 17 autosomes, 1 large X chromosome, and 127 unplaced scaffolds using PacBio HiFi and Hi-C sequencing. With the availability of this new genome, we draw broad comparisons of repeat composition and macrosynteny among available spider genomes, spanning 300 million years of spider evolution.

Results and Discussion

Raw Data

The PacBio HiFi long-read sequencing produced circular consensus sequencing (CCS) reads from eight SMRT cells, generating 17,983,734 reads comprising 266,088,584,498 base pairs of data, approximately 41× sequence coverage relative to the final assembly. CCS reads had an average length of 14,796.07 bp and a max length of 50,115 bp. The phase genomics Hi-C sequencing yielded 553,425,642 reads for a total of 83,013,846,300 bp, corresponding to ~12.8× sequence coverage of the final assembly. All data generated for this project, including the final genome assembly, PacBio HiFi CCS reads, Hi-C reads, and mitochondrial genome (PV987586), have been deposited on NCBI under the BioProject ID: PRJNA1269020.

Genome Assembly, Quality, and Annotation

The Jellyfish and GenomeScope analyses estimated that the genome size of *A. marxi* was 4.6 Gb (haplotype) with a heterozygosity of 0.678% (Fig. 1a), greatly underestimating the final genome size. The Hifiasm primary assembly was estimated at 7 Gb (haploid) that consisted of 1,744 contigs with an N50 of 75.2 Mb (75,222,789), an L50 of 32, and L90 of 111. Merqury estimated the completeness for hap1 and hap2 were 78.5% and 82.5%, with the combined diploid assembly achieved an overall completeness of 98.1% (Fig. S1). Merqury estimated that the consensus quality value or QV scores of hap1 and hap2 were 63.36 and 64.20, while the combined diploid assembly achieved an overall completeness of 63.77. The removal of haplotigs with Purge_dups greatly reduced the number of contigs in the primary assembly from 1,744 to 256 and reduced the primary genome size to 6.5 Gb (haploid). Additionally, the purging of the genome subsequently increased the N50 (79.4 Mb), average contig length and smallest length, while also improving the L50 and L90. The haplotypic purging of the genome led to a slight increase in single-copy Arachnida (odb10; 2,934 genes) BUSCOs (86.6% to 87.3%) and a slight decrease in the number of duplicated BUSCOs (10.1% to 9.3%), with the overall complete BUSCOs being very similar 96.7% versus 96.6%. Using YaHS with two rounds of scaffolding led to a more contiguous genome; however, no additional improvement was seen after three rounds.

The scaffolded genome comprises 18 chromosomal scaffolds that make up 99.7% of the total genome, along with 127 unplaced scaffolds, totaling 6.5 Gb (6,491,949,952 bp). Scaffolds are named in order of size from largest to smallest. During manual curation, three edits were made to the assembly: (i) an inversion of chromosome 4 at the first 95 Mb; (ii) a 2 Mb region immediately after the inverted region of chromosome 4 was moved to the end of chromosome 6; and (iii) a 37 Mb inversion near the beginning of chromosome 6 (see uncorrected scaffolds in Fig. S2). The remaining, much smaller 127 scaffolds, which varied in size from 1 kb to 4 Mb, could not be unambiguously placed in the 18 chromosomal scaffolds (Fig. 1b). The average coverage across the 18 chromosomes was 40×, except for three outliers. The second largest chromosome had roughly half the coverage (23×) compared to the average suggesting that this is likely an X chromosome and is herein referred to as such. The higher-than-average coverage in chromosomes 11 (48×) and 16 (63×) is driven by localized regions likely where the assembly graphs collapsed contigs. HiFi coverage of the 145 scaffolds is reported in Table S1, Tab 1.

NCBI FCS-GX did not identify any regions of contamination in our genome; however, NCBI FCS Adaptor revealed 31 bp of adaptor in chromosome 13 which was excised from the genome. The final *A. marxi* genome consists of 17 autosomes and 1 X chromosome, with the X chromosome being the second largest

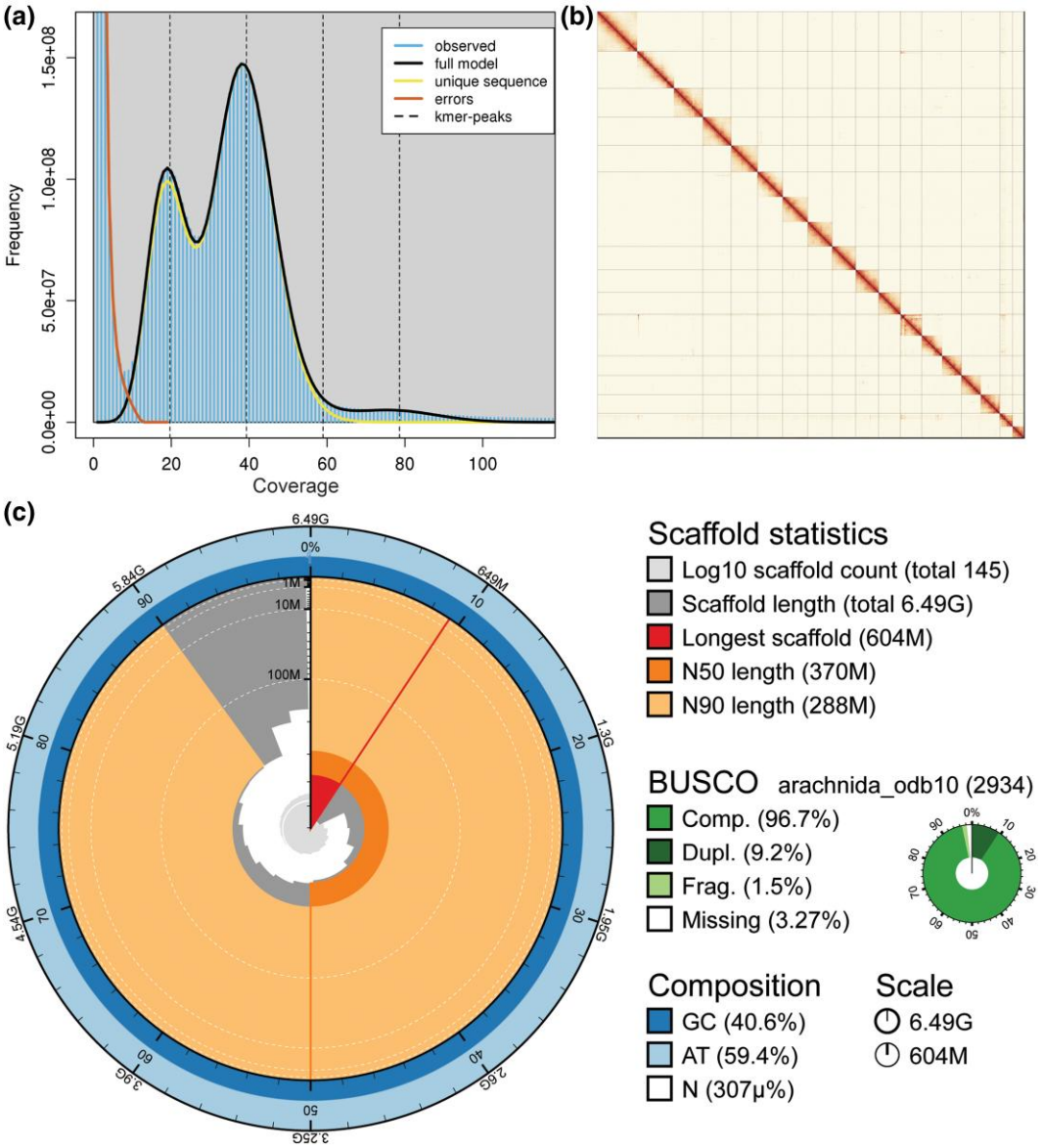

Fig. 1. *Aphonopelma marxi* genome assembly. a) GenomeScope and Jellyfish output of PacBio HiFi CCS reads using *k*-mer 31 showing detectable heterozygous and homozygous peaks at ~20× and ~40× coverage. b) Manually curated Hi-C contact map using PretextView. More intense colors show greater associations of Hi-C reads along 18 chromosome scaffolds, which are ordered largest to smallest. Scaffold 2 was identified as an X chromosome from lower intensity of color reflective of approximately half coverage. c) A snail plot of the *A. marxi* assembly along with genome statistics, Arachnida (odb10; 2934 genes) BUSCO percentages, and genome composition.

chromosome (Fig. 1b and c). Additionally, the final genome comprises 6.5 Gb (6,491,949,921 bp) with an N50 of 370 Mb (370,027,674 bp), an L50 of 8, an L90 of 16, and a GC content of 40.6%. BUSCO analysis of the final genome suggests the genome is highly complete: complete = 96.7%, single = 87.5%, duplicated = 9.2%, fragmented =1.5%, missing = 1.8%, $n = 2934$. Merqury identifies that the assembly has a completeness of 82.95 and a QV score of 64.94. Compared to the benchmark standards proposed by the Earth BioGenome Project (EBP), our primary haploid *A. marxi* assembly exceeds the baseline for reference genomes (QV ≥ 40) and approaches telomere-to-telomere (T2T)–level accuracy (QV > 60). Genome completeness, assessed via BUSCO (96.7%) and Merqury (~83%), meets EBP targets for gene and *k*-mer representation, with the slightly lower *k*-mer completeness reflecting the haploid nature of the assembly. Additionally, chromosome-scale contiguity metrics (N50 370 Mb, L50 8) are consistent with EBP recommendations for highly contiguous reference genomes,

confirming that the assembly is both structurally contiguous and functionally comprehensive.

Earl Grey identified 78.77% of the *A. marxi* genome as repetitive. These repetitive elements and their respective proportions comprise DNA = 41.26%, rolling circle = 0.06%, Penelope-like = 4.84%, LINEs = 9.53%, SINEs = 0%, LTRs = 6.19%, other = 1.82%, and unclassified as 15.03%. MitoHiFi recovered and assembled the mitochondrial genome that was 13,891 bp, with 13 protein-coding genes, 2 rRNAs, and 22 tRNAs annotated.

Comparative Genomics

When comparing estimated repeat content of spider genomes from 15 families across the Order (Table 1 and Fig. 2b), we find that mygalomorphs have more repetitive genomes compared to their more diverse sister lineage, the araneomorphs. Among the four mygalomorph genomes available (i.e. from two families: Theraphosidae and Macrothelidae), their genomes range from 75% to 80% repetitive, with DNA transposons comprising most of this repeat content (33% to 41%), followed by unclassified repeats (19% to 24%), then LINEs (8% to 14%). Within the araneomorphs, repeat content is more varied ranging from 36.9% to 76.3%, with DNA transposons, LTRs, and unclassified repeats making up the bulk of the repetitive content. Our results are consistent with recently published results using some of the same genomes to compare repeat content (Papadopoulos et al. 2025). The overall high repeat content found in these spider genomes is consistent with the growing evidence for shared whole genome duplication event in the common ancestor of the Arachnopulmonata (Clarke et al. 2015, Di et al. 2015, Sharma et al. 2015, Schwager et al. 2017, Shingate et al. 2020, Sharma 2023, Kulkarni et al. 2025) of which spiders are members. We also observed that genome size and repeat content were correlated but with some exceptions. Within the Araneoidea lineage sampled in our analysis (Araneidae, Nephilidae, Linyphiidae, Tetragnathidae, Theridiidae), there was a clear trend of decreases in genome size and repeat content, supported by the strong positive correlation observed across the available genomes (Pearson's $r = 0.74$, $P = 1.2 \times 10^{-4}$). This pattern could suggest that repetitive elements have been selectively lost or removed, rather than lost randomly or repurposed through neofunctionalization, which might occur if repeat content decreased but genome size remained stable. This decoupling is illustrated by comparing *Amaurobius ferox* and *Salticus scenicus*, which exhibit similar repeat contents (~74% vs. 76%) despite substantially different genome sizes (3.6 Gb vs. 5.2 Gb, respectively; Fig. 2a and b).

Interestingly, the broad overall composition of repetitive elements in mygalomorphs appears to be quite similar despite ~170 Ma of divergence between the two families (Macrothelidae and Theraphosidae) (Opatova et al. 2020). An exciting hypothesis based on these results could suggest the constrained evolution of mygalomorphs based on limited niche space (Wilson et al. 2023) could also be represented in overall genomic composition. However, it is uncertain whether these patterns will hold with the sampling of broader phylogenetic diversity across mygalomorphs or if similar levels of variation exist as seen in the araneomorphs. Additionally, as all spider genomes we examined contained a significant portion of unclassified repetitive elements (11.65% to 50.28%), this suggests that repetitive elements do not share sufficient homology with the current curated datasets to be confidently classified. Therefore, the composition could change quite drastically with future curation of arachnid transposable elements (TEs) and subsequent reanalysis of the genomes.

When assessing genome quality and completeness, the recovery of lineage-specific BUSCOs is a frequently used indicator (Waterhouse et al. 2018, Manni et al. 2021); however, we find evidence for a lineage-specific discrepancy in spiders. When reporting the proportion of completed BUSCOs, mygalomorph spiders have a consistently lower proportion (95.4 to 96.7) than araneomorphs (up to 98.4%) (Table 1 and Fig. 3a and b). This difference does not appear to be explained by sequencing coverage with mygalomorph samples having a range of sequencing effort of 20×, 40×, 62×, and 114× coverage. We believe this overall lower recovery of BUSCOs is due to mygalomorphs either having missing BUSCOs or BUSCOs that are derived and only detectable in araneomorphs and Mesothelae. Examination of the missing BUSCOs (Table S1, Tab 2) revealed that 28 orthologs were reported as absent from the four mygalomorph genomes, representing 0.94% of the Arachnida odb10 dataset (28/2953). Notably, the spider representatives used in the construction of the Arachnida odb10 lineage within BUSCO include only araneomorph species *Stegodyphus mimosarum* and *Parasteatoda tepidariorum*, rather than any mygalomorph taxa. This phylogenetic bias may partially explain the lower BUSCO completeness observed in the divergent mygalomorph as well as hypochilid genomes. Interestingly, the most phylogenetically derived Mesothelae spider performed slightly better than the mygalomorphs, suggesting that the observed differences are not strictly correlated with divergence. These results indicate that the Arachnida odb10 lineage may underrepresent orthologs conserved in some early-diverging spider lineages, and potential lineage-specific biases should be considered when interpreting BUSCO completeness estimates.

Table 1 Genome statistics, BUSCO percentage scores, and repeat composition percentages of genomes compared in this study. NR = Not reported. ERGA = European Reference Genome Atlas, IZCAS = Institute of Zoology Chinese Academy of Science, DTOL-WSI = Darwin Tree of Life Wellcome Sanger Institute.

Sub/infraorder	Family	Species	NCBI_accession	Genome size (Gb)	Chr no.	X?	Total scaffs	Cov.	N50	L50	GC %	BUSCO_arachnid_2953					Earl Grey Repeat composition									Source
												Comp.	Sing.	Dup.	Frag.	Miss	DNA	Rolling Cir.	Pene.	LINE	SINE	LTR	Other	Unclass.	Non-rep.	Repeat	
Mygalomorphae	Macrothelidae	*Macrothele yani*	GCA_039090855.1	6.7	46	NR	432	114×	156.3 Mb	19	40	95.5	89.4	6.1	2.1	2.4	34.26	0.65	4.46	14.42	0	1.74	2.25	22.21	19.97	80.03	You et al. (2024)
Mygalomorphae	Macrothelidae	*Macrothele cretica*	GCA_964417605.1	3.9	47	11 X	561	62×	95 Mb	19	40.5	96	89.3	6.7	1.5	2.5	38.05	0.11	3.5	8.05	0.23	2.16	3.47	21.06	23.32	76.68	ERGA
Mygalomorphae	Theraphosidae	*Aphonopelma marxi*	GCA_054742115.1	6.5	18	1 X	145	40×	370 Mb	8	40.6	96.7	87.5	9.2	1.5	1.8	41.26	0.06	4.84	9.53	0	6.19	1.82	15.03	21.23	78.77	This study
Mygalomorphae	Theraphosidae	*Chaetopelma lymberakisi*	GCA_964291345.1	5	33	NR	2068	20×	161.8 Mb	14	41.5	95.4	88.9	6.5	1.4	3.2	33.88	0.004	3.9	11.17	0.001	4.07	4.22	17.74	24.97	75.03	ERGA
Araneomorphae	Hypochilidae	*Ectatosticta davidi*	…	2.1	15	NR	1600	89×	146 Mb	6	36	96.1	89.9	6.2	1.1	2.8	13.09	1.1	8.74	6.92	0	2.68	1.44	37.63	28.35	71.65	Fan et al. (2023)
Araneomorphae	Eresidae	*Stegodyphus mimosarum*	GCA_044660205.1	2.9	16	2 X	16	30×	195.6 Mb	7	34	98.2	93.5	4.7	1.1	0.7	20.01	0.45	1.16	4.91	0.12	9.44	0.1	27.65	36.12	63.88	Ma et al. (2025)
Araneomorphae	Eresidae	*Stegodyphus tentoriicola*	GCA_044658465.1	2.9	14	2 X	14	30×	237.6 Mb	6	33.5	97.8	92.2	5.6	1.3	0.9	19.14	0.2	0.86	6.06	0.05	11.06	1.23	28.46	32.89	67.11	Ma et al. (2025)
Araneomorphae	Uloboridae	*Octonoba sinensis*	GCA_038502835.1	1.3	9	NR	17	30×	139.9 Mb	4	32.5	95.2	90.6	4.6	0.9	3.9	9.37	0.18	1.04	1.52	0.26	3.25	0.4	41.45	42.48	57.52	IZCAS
Araneomorphae	Amaurobiidae	*Amaurobius ferox*	GCA_951213105.1	3.6	23	3 X	288	31×	153.4 Mb	11	33	98	92.8	5.2	0.6	1.4	15.82	0.38	4.33	4.62	0.32	3.82	2.78	44.22	23.67	76.33	Henriques (2024)
Araneomorphae	Agelenidae	*Eratigena atrica*	GCA_965122215.1	4.9	22	2 X	474	38×	231.9 Mb	10	32	98	93.1	4.9	0.9	1.1	14.05	0.16	3.5	3.45	0	5.32	3.56	35.12	34.79	65.21	DTOL-WSI
Araneomorphae	Salticidae	*Salticus scenicus*	GCA_963930645.1	5.2	15	NR	3113	28×	342 Mb	7	30	97.3	91	6.3	1	1.7	10.41	1.02	2.54	2.02	0.13	6.22	2.1	50.28	25.52	74.48	DTOL-WSI
Araneomorphae	Lycosidae	*Pardosa pseudoannulata*	GCA_032207245.1	2.4	15	NR	1958	180×	170 Mb	7	29	96.5	90.8	5.7	1.6	1.9	11.21	0.2	0.13	2.77	0.002	3.73	5.62	33.23	43.06	56.94	Yu et al. (2024)
Araneomorphae	Theridiidae	*Latrodectus elegans*	GCA_030067965.1	1.6	14	NR	153	60×	114.3 Mb	7	28	98.4	93.8	4.6	0.5	1.1	13.74	0.15	0.19	6.94	0.39	3.79	0.1	11.65	63.01	36.99	Wang et al. (2022)
Araneomorphae	Theridiidae	*Parasteatoda tepidariorum*	GCF_043381705.1	1.1	12	2 X	684	100×	93.9 Mb	6	29.5	98	93.6	4.4	0.7	1.3	5.9	0.23	0.77	1.79	0.15	0.65	0.26	35.4	54.8	45.2	Zhu et al. (2023)
Araneomorphae	Tetragnathidae	*Tetragnatha montana*	GCA_963680715.1	0.7	13	2 X	82	26×	61.1 Mb	7	33.5	97.4	91.7	5.7	0.7	1.9	3.39	0.07	0.16	3.41	0.33	2.76	1.07	35.9	52.88	47.12	McGregor et al. (2024)
Araneomorphae	Linyphiidae	*Hylyphantes graminicola*	GCA_023701765.1	0.9	13	NR	103	144×	77.1 Mb	6	32.5	97.7	82.7	15	0.5	1.8	4.93	0.13	0.05	2.4	0.36	2.78	0.6	40.14	48.57	51.43	Zhu et al. (2022)
Araneomorphae	Linyphiidae	*Troglohyphantes excavatus*	GCA_963932185.1	1	13	NR	134	29×	74.9 Mb	7	32	98.1	93.1	5	0.7	1.2	4.03	0.27	0.38	2.72	0.61	2.55	8.38	38.11	42.91	57.09	Pavelk et al. (2025)
Araneomorphae	Nephilidae	*Trichonephila antipodiana*	GCA_050495765.1	2.3	13	NR	377	100×	172.9 MB	6	29	97.7	92.8	4.9	1	1.3	22.1	2.73	1.09	2.68	0.34	5.26	1.23	25.21	39.3	60.7	Fan et al. (2023)
Araneomorphae	Araneidae	*Argiope bruennichi*	GCF_947563725.1	1.8	13	2 X	29	28×	139.1 Mb	7	29.5	98.2	92.8	5.4	0.6	1.2	8.35	0.55	0.1	3.14	0.04	1.65	2.08	27.72	56.33	43.67	Crowley et al. (2023)
Araneomorphae	Araneidae	*Leviellus thorelli*	GCA_965183905.1	2.2	13	NR	130	40×	167.1 Mb	7	34.5	96.5	90.8	57	1.6	1.9	21.26	2.23	0	3.44	0	5.48	1.52	30.33	35.72	64.28	ERGA
Mesothelae	Heptathelidae	*Ryuthela nishihirai*	GCA_038380435.1	3.1	NR	NR	1711	39×	77.1 Mb	15	38.5	97.2	89.8	7.4	1.2	1.6	14.82	0.005	0.94	18.45	0.67	2.11	3.43	25.68	33.76	66.24	Schöneberg et al. (2025)

When comparing GC% content between *A. marxi* and the other 20 spider genomes, we find mygalomorphs ($n = 4$) have higher GC content ranging 40% to 41.5%, while araneomorphs ($n = 16$) possess 29.5% to 34.5%; the only available Mesothelae had 38.5%. GC content is expected to increase in lineages that have higher recombination rates per time unit (Spencer et al. 2006, Romiguier et al. 2010, Romiguier and Roux 2017, Botero-Castro et al. 2017), a finding that appears to be the opposite case in some spiders. Because of their longer generation times, mygalomorph spiders presumably have a lower recombination rate when compared to araneomorph spiders, yet mygalomorphs have a higher GC content, suggesting other factors may be at play. Brewer et al. (2014) highlighted the lower GC content in the available arachnid genomes of the time; however, the broader patterns and evolutionary consequences (if any) of GC content do not yet appear to have been deeply examined in spiders as in other lineages (Galtier et al. 2009, Weber et al. 2014, Kyriacou et al. 2024, Clessin et al. 2025). What we do know is that higher GC-rich genomic regions are also often associated with higher gene density and genomic organization. But perhaps mygalomorph spiders and their high GC content could be linked to adaptation in high-temperature environments because of its higher thermal stability due to an additional hydrogen bond—a feature correlated to the stability of DNA and RNA transcripts (see Šmarda et al. 2014 and Hu et al. 2022). As the tropics are the dominant region for mygalomorphs, this could have been a highly favored trait for mygalomorph evolution in the Carboniferous and Triassic periods. However, with limited sampling and until further examination of these genomes is undertaken, we are cautious to overinterpret the cause of this pattern.

Being able to identify homologous genomic regions brings evolutionary biologists a step closer to comparing how genomes change through time, tracing fission/fusions, and recombination events. Previous arachnid genomic studies have compared macrosynteny between a few closely related species (Zhu et al. 2022, Miles et al. 2024, Castellano et al. 2025, Wang et al. 2025) or more broadly across arachnids (Kulkarni et al. 2025); however, our study is the first to examine more broadly across spiders. Through identification of macrosynteny between spider genomes using the single-copy orthologs from the BUSCO analysis, we identified putatively homologous chromosomes (or regions) across 300 Ma of evolution within the group to reveal several key findings (Fig. 4). Because many of the spider genomes were sequenced using female specimens, X chromosomes could not be identified using sequencing coverage, leaving only nine lineages with sex chromosome/s identified from the 20 used in our analyses. However, we were able to tentatively identify likely X chromosomes by linking syntenic regions. For example, the single X chromosome in *A. marxi* shows synteny with three of the *C. lymberakisi* chromosomes (5, 14, and 30) suggesting these could be three separate X chromosomes in the female *C. lymberakisi* genome. In a more complicated example whereby comparing synteny between *A. marxi* (1 X chromosome) and *Macrothele cretica* (11 X chromosomes), we find homology between X chromosomes as well as possible fusion/fission of X and non-X chromosomes. In *M. cretica*, we find eight X chromosomes showing homology to the *A. marxi* X chromosome, with X7 and X11 showing no significant matches; however, X8 and part of X6 appear to have homology with non-X chromosomes in *A. marxi*. The possible fusion of X and non-X chromosomes can be again seen when comparing synteny between *A. marxi* and the hypochilid spider (*Ectatosticta davidi*), where possible fusion between X and non-X chromosomes occurred.

In the context of current spider classification, there are distinct patterns within and between groups. In the much more diverse araneomorphs, the sampled lineages possess a lower number of chromosomes (9 to 23) compared to the mygalomorphs (18 to 47). While structural rearrangements between homologous araneomorph chromosomes are evident, the relatively lower chromosome number of this group identifies much less fusion/fission of chromosomes. However, this is not without exceptions, as can see much fusion/fission when comparing the *Pardosa pseudoannulata* (a wolf spider) and *Latrodectus elegans* (black widow spider). On the other hand, when comparing groups with more varied karyotype number such as the mygalomorphs (18 to 47), we see many structural rearrangements and fission/fusions, reflecting the large changes in karyotype number. For example, chromosome 1 in *A. marxi* appears to be a fusion of *C. lymberakisi* chromosomes (or parts thereof) 2, 3, 4, 11, 23, and 31. These large genomic structural changes in relatively morphologically conserved groups such as the mygalomorphs require further investigation to see if such patterns might be associated with other aspects of their biology, in turn influencing their evolution. However, given the limited number of mygalomorphs available, many more genomes are needed to better understand the patterns (if any) of genomic change across this clade.

To aid in better understanding spider evolution, we present a high-quality, chromosomal genome for *A. marxi*. This is the first available genome for the most well-studied genus of tarantula and only the fourth chromosomal level genome for mygalomorph spiders. Compared to other important lineages on this planet (e.g. the vertebrate bias), arachnid genomics is still in

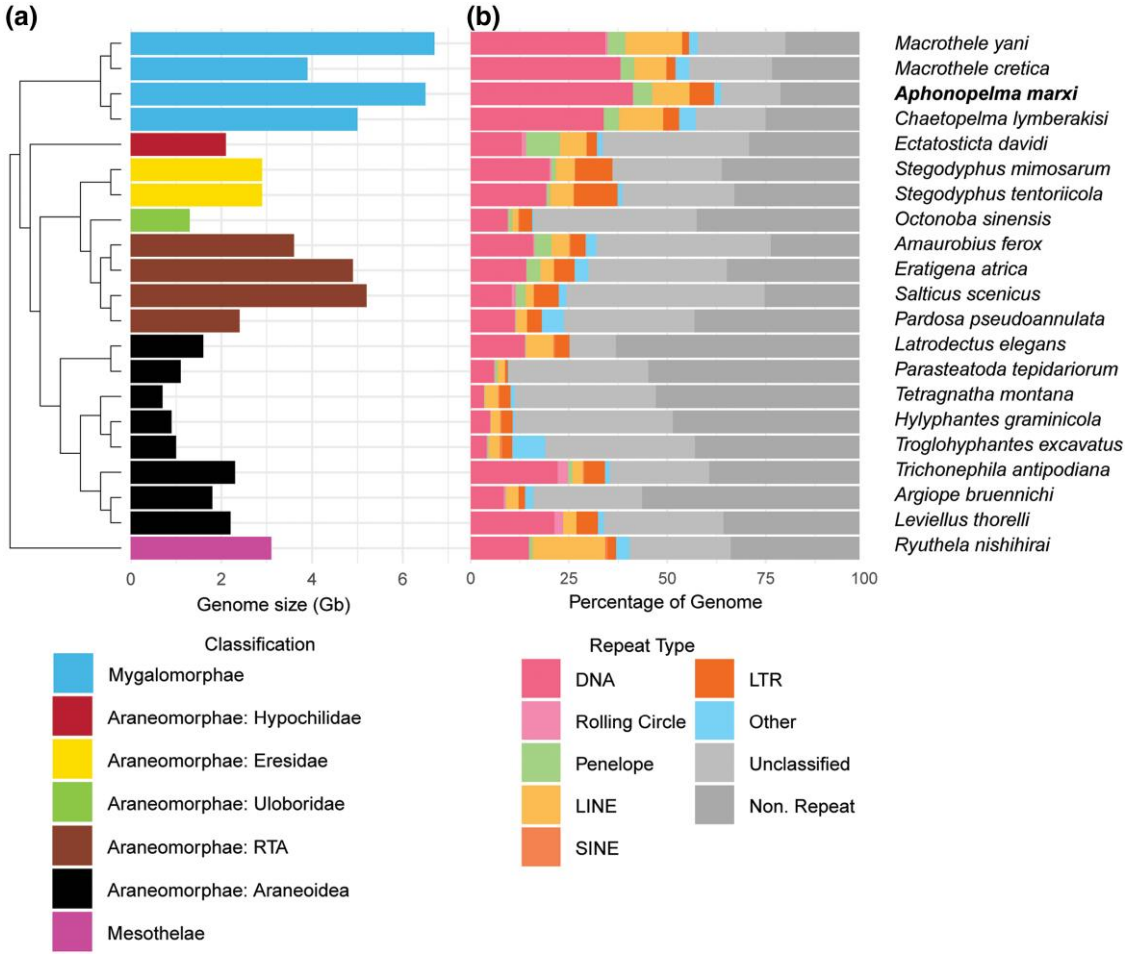

Fig. 2. Comparative visualization of spider genomes in the context of classification and evolutionary relationships. a) Variation in spider genomes showing genome size colored by taxonomic classification. b) Composition of repeat content identified using the Earl Grey pipeline. *Aphonopelma marxi* is designated in bold. Phylogeny is leveraged from supported clades in Wheeler et al. (2017) and Schoneberg et al. (2025).

its infancy; however, the continued addition of high-quality spider genomes provides crucial data that helps further push the knowledge boundaries of arachnid evolutionary biology.

Materials and Methods

Sampling

One adult male *A. marxi* (Simon 1891) was collected from near Happy Jack, Coconino County, Arizona, United States of America, on October 29, 2022, and was brought live to the University of Idaho (UI), Moscow, Idaho, United States of America. The specimen was given the identification code APH_5344 and flash frozen in liquid nitrogen, then stored in conical tubes at −80 °C at the UI prior to genomic extractions. The specimen was identified using the revised taxonomy of *Aphonopelma* proposed by Hamilton et al. (2016).

Genomic Extractions and Sequencing

To extract genomic DNA, we used the PacBio (previously Circulomics) Nanobind tissue kit, following manufacturer's protocol. Leg tissue (15 to 20 mg) was homogenized using the TissueRuptor (Qiagen). To evaluate genomic extraction success, the amount of DNA was measured on a Qubit 4 Fluorometer (Thermo Fisher) in triplicate. DNA length and integrity was evaluated using a 1% agarose gel run at 80 V for 90 min. DNA samples were stored at 4 °C prior to library preparation.

PacBio HiFi library preparation and sequencing were completed at the University of Idaho Genomics Core. After intake quality control (QC) (Fragment Analyzer, NanoDrop, and Qubit), samples were pooled then sheared on Megaruptor III. DNA was concentrated after shearing to size-select using a BluePippin (Sage Science) and 0.75% dye-free cartridges targeting a mixture of 12 to 50 kb and 10 to 50 kb. The PacBio Template Prep Kit 3.0 was used for end-repair/prep, a-tailing, adaptor

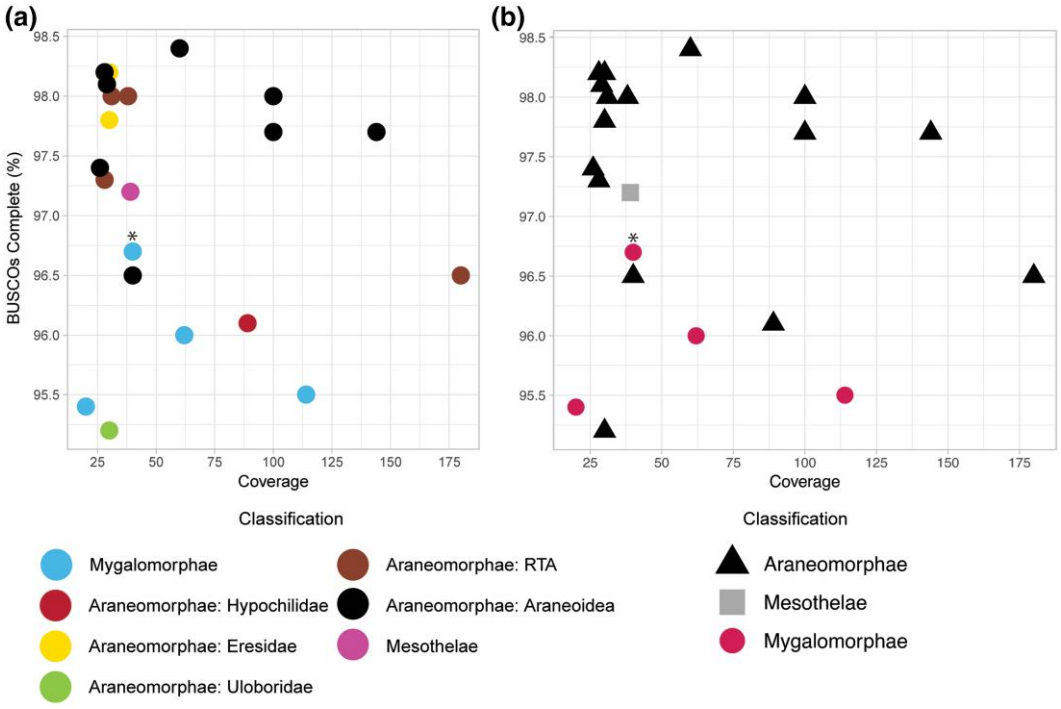

Fig. 3. Proportion of BUSCOs by classification. a) Percentage of complete Arachnida (odb10; 2,934 genes) BUSCOs versus sequencing coverage with points colored by classification. b) Colored by higher classification showing mygalomorphs with overall lower complete BUSCO proportions. *Aphonopelma marxi* is designated asterisk.

ligation, and nuclease treatment. Binding Kit 3.2 was then used to anneal primer, bind polymerase, and then dilute that complex and additionally add the control complex. Sequencing was performed on a PacBio Sequel II using the sequencing kit 2.0, for 30 h, with 2 h of pre-extension time, and with adaptive loading on targeting 0.85 P1 + P2.

Frozen leg and cephalothorax tissue were then sent to Phase Genomics (PG), Seattle, who prepared a Hi-C library (Lieberman-Aiden et al. 2009, Van Berkum et al. 2010) using their Proximo kit v4.5. PG performed QC of the Hi-C library by generating a small number of reads using an Illumina iSeq100 and aligning those reads to the temporary assembly. After successful QC, PG sent the library to Azenta Life Sciences for deep, paired-end 150 bp sequencing using an Illumina NovaSeq X.

Genome Assembly and Repeat Annotation

The raw subreads from the eight 8 M SMRTBell cells were processed using CCS under default settings with SMRT link v12. During CCS read generation, adapters were automatically removed and with reads being quality filtered based on minimum passes and predicted accuracy thresholds. The resulting HiFi CCS reads were then polished with DeepConsensus (Baid et al. 2023) to improve consensus accuracy. Using the *k*-mers from the CCS HiFi reads, Jellyfish v2.3.0 (Marçais and Kingsford 2011) and GenomeScope v2.0 (Vurture et al. 2017) were employed to estimate genome size, coverage, and heterozygosity, as well as assess whether both haploid and diploid peaks were present. For both programs, we used *k*-mer = 31. To produce a primary draft assembly, we used Hifiasm v0.19.8 (Cheng et al. 2021) under default settings. We then evaluated the completeness and quality of the draft assembly using reference-free, *k*-mer–based Merqury (Rhie et al. 2020). Based on the larger draft assembly size than the initial estimates and likely high repeat content, we used a larger *k*-mer of 51 for Merqury to improve resolution in repetitive regions. Identification and removal of haplotigs from the primary assembly were performed using Purge_dups v1.2.6 (Guan et al. 2020) under default settings. To scaffold our purged assembly with YaHS v1.2.2 (Zhou et al. 2023), we aligned the Hi-C reads to produce the required BAM file using minimap2 v2.26 (Li 2018, 2021) and SAMtools v1.13 (Li et al. 2009, Danecek et al. 2021). YaHS was trialed with one, two, and three rounds of scaffolding to evaluate whether any improvements could be made with additional rounds of scaffolding. Hi-C contact maps were prepared and viewed using the PretextMap and PretextView (https://github.com/sanger-tol/PretextView). A HiFi coverage track was generated by aligning the HiFi reads to the genome using BEDtools v2.31.1 (Quinlan and Hall 2010, Quinlan 2014) *genomecov*, which was then integrated

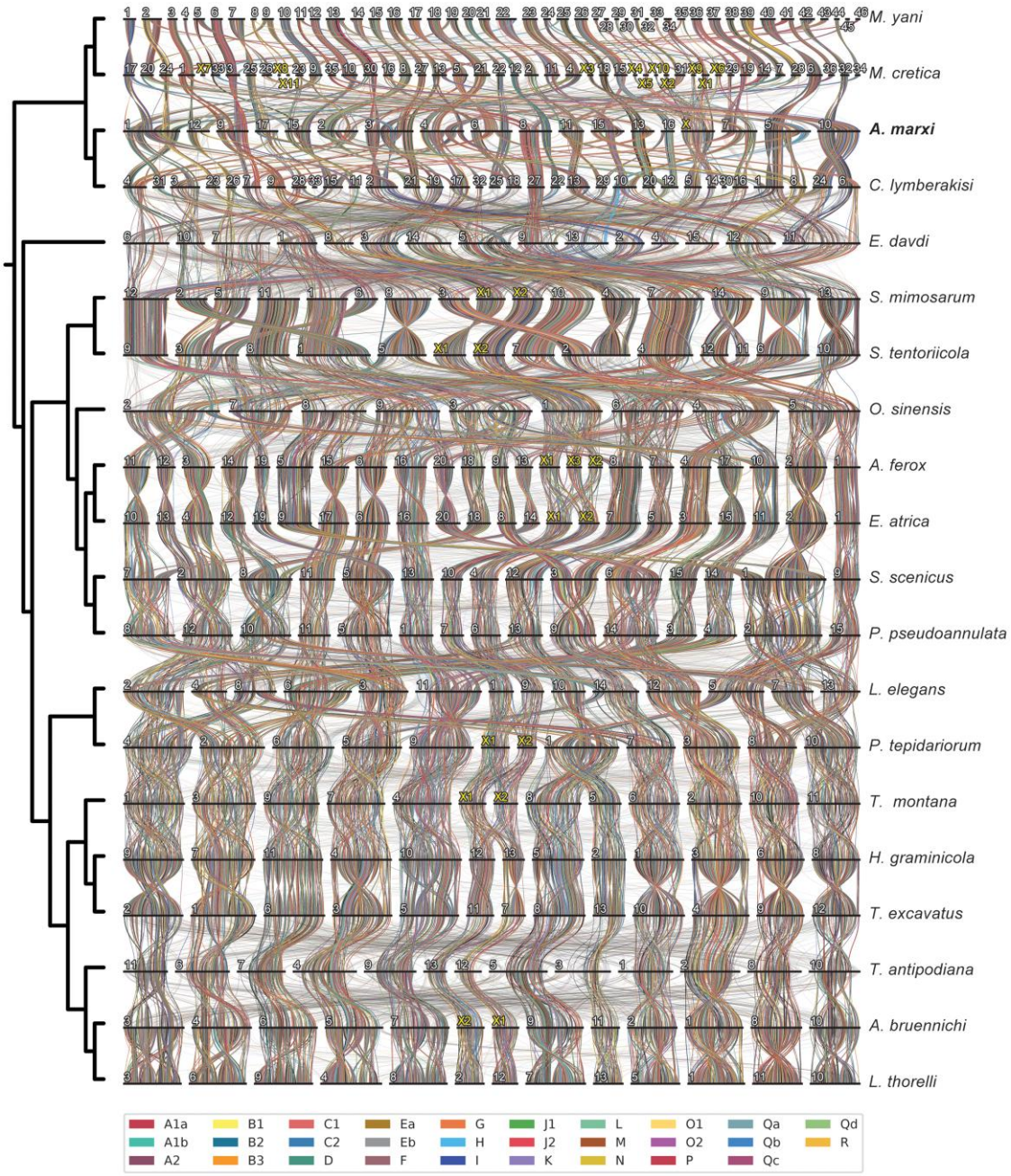

Fig. 4. Macrosynteny using single-copy BUSCOs and odp pipeline. Links between genomes colored by Bilateria-Cnidaria-Sponge ancestral linkage groups as seen in bottom of figure (see Simakov et al. 2022). Non-gray links between chromosomes are significant ($P \leq 0.05$) based on Fisher's exact test of reciprocal best hits. Gray links represent non-significant associations ($P > 0.05$). Black bars represent chromosomes, each being numbered based on their ID from publicly available databases. Chromosomes with names in yellow were identified as X chromosomes by the authors of the respective genomes. *Aphonopelma marxi* is designated in bold. Phylogeny is leveraged from supported clades in Wheeler et al. (2017) and Schoneberg et al. (2025).

into the Pretext map with PretextGraph. Unambiguous scaffold mis-joins or inversions were manually corrected in Pretext using the coverage and Hi-C associations of the contact map before producing an AGP file. Due to the large size of the genome and presence of some relatively tiny scaffolds, the AGP file was corrected using the AGPCorrect python script provided from PretextView. The corrected AGP output and FASTA file were then used to produce the corrected assembly using agptools (https://github.com/WarrenLab/agptools) with the command *assemble*. To assess if any contamination was present in the *A. marxi* genome, we used the NCBI FCS-GX

v0.5.5 (Astashyn et al. 2024) through Galaxy (Afgan et al. 2016) with the following settings: Complete GX database, GX Division, Animals—Insects. FCS-Adaptor v0.5.5 was employed using a Singularity container to check for adaptor contamination. In cases where contamination was identified, they were excised from the assembly. The completeness and quality of our genome were then evaluated using Benchmark Universal Single Copy Orthologs v5.4.3 (Manni et al. 2021) and the Arachnida (odb10; 2,934 genes) database to report the proportion of single, duplicated, fragmented, and missing BUSCOs. A snail plot with genome statistics and BUSCO proportions was produced using BlobToolKit (Challis et al. 2020). Final assembly quality and completeness were again checked with Merqury. We report final assembly statistics such as N50, L50, and scaffold length, using the asmstats script (https://github.com/mcshane).

To identify repetitive elements and TE activity within the *A. marxi* genome, we utilized Earl Grey v6.0.1 (Baril et al. 2024). The automated Earl Grey pipeline employs commonly used alignment and annotation tools including RepeatMasker (http://www.repeatmasker.org), Repeat Craft (Wong and Simakov 2019), Dfam (Storer et al. 2021), RepeatModeler (Flynn et al. 2020), RepeatScout (Price et al. 2005), Blast + (Camacho et al. 2009), MAFFT (Katoh and Standley 2013), TrimAl (Capella-Gutiérrez et al. 2009), Tandem Repeat Finder (Benson 1999), RECON (Bao and Eddy 2002), and LTR_FINDER (Xu and Wang 2007, Ou and Jiang 2019), as well as other open-source code from EMBOSS (Rice et al. 2000). Functional annotation of the genome was beyond the scope of this study but will be investigated in the future. To assemble the mitochondrial genome of *A. marxi*, we used the Docker version of MitoHiFi v3.2.2 (Uliano-Silva et al. 2023). We first downloaded the *Brachypelma albiceps* mitogenome using the findMitoReference command, as it is the closest related species (as shown by Turner et al. 2018) with an available mitogenome (NC_062668.1). Annotation was performed using the *B. albiceps* annotations in Geneious Prime 2025.1.3 (https://www.geneious.com). All bioinformatics were performed on the University of Idaho HPC unless otherwise detailed. Scripts and code used for this study are available on a GitHub repository (https://github.com/ethanbriggs/Aphonopelma_genome).

Comparative Genomics and Macrosynteny

To compare our genome assembly to other publicly available spider genomes, we downloaded 20 samples (19 from NCBI and 1 from ScienceDB Digital Repository) spanning 15 families (Table 1) (O'Leary et al. 2024). Genomes were chosen to span the available phylogenetic diversity, as well as the most contiguous genomes when more than one species from a family was available; there are approximately 40 spider chromosomal genomes on NCBI datasets. Only one genome (*Ryuthela nishihirai*) was designated as "scaffold" was included for comparison given its important phylogenetic position—the Mesothelae are sister to all other known spiders. To compare genomes, we report genome size, chromosome number (when reported), number of X chromosomes (when reported), total scaffolds, coverage, N50, and L50 values directly from the NCBI Datasets genome portal. To compare BUSCO scores, we analyzed each genome using the Arachnida (odb10; 2,934 genes) database. To consistently compare the amount and composition of repetitive elements between the genomes, we again used Earl Grey with identical settings for all genomes analyzed. To compare and visualize macrosynteny between spider lineages, we employed odp v0.3.0 (Schultz et al. 2023). Using the 19 other spider lineages (excluding *R. nishihirai* due to no chromosome designation), we aligned the recovered BUSCO single-copy orthologs to their respective genomes using minimap2, converting SAM files to BAM then BED files. The BED files were formatted to be consistent with the input chrom files needed to run odp. The reciprocal-best BLASTp hits were used to identify macrosyntenic chromosomes between lineages by performing Bonferroni-corrected one-sided Fisher's exact tests (Simakov et al. 2020). We used in-built tools in odp v0.3.0 to visualize syntenic patterns (i.e. Oxford dot plots and ribbon plots). BUSCOs were colored using the Bilateria-Cnidaria-Sponge ancestral linkage groups (BCnS-ALGS) as defined in Simakov et al. (2022). Phylogeny is adapted from supported clades in Schoneberg et al. (2025) who used BUSCOs to estimate their phylogenies, as well as Wheeler et al. (2017) for araneomorph taxa not represented in Schoneberg et al. Figures 2 and 3 in this study were produced with R v4.1.1 in RStudio v2023.06.2 (R Core Team 2023) using the R packages: ggplot2 v3.5.2 (Wickham 2016), tidyverse v2.0.0 (Wickham et al. 2019), ggtree v3.8.2 (Yu et al. 2017), and ape v5.8.1 (Paradis et al. 2004).

Supplementary Material

Supplementary material is available at *Genome Biology and Evolution* online.

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Author Contributions

Ethan J. Briggs (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing—original draft, Writing—review & editing), Andrea J. Noble-Stuen (Investigation, Writing—review & editing), and Chris A. Hamilton (Conceptualization, Investigation, Funding acquisition, Supervision, Visualization, Writing—review & editing)

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Data Availability

All sequencing data generated as part of this study have been deposited on NCBI under BioProject ID: PRJNA1269020 and BioSample Accession: SAMN48 773856. Final genome can be found on NCBI Dataset as Genome assembly ASM5474211v1. Scripts and code used in this study have been placed in a public GitHub repository (https://github.com/ethanbriggs/Aphonopelma_genome).

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